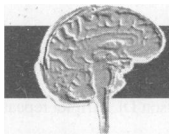

Neurotrophic Factors and Neurologic Disease

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Discovered only 40 years ago, nerve growth factor is the prototypic neurotrophic factor. By binding to specific receptors on certain neurons in the peripheral nervous system and brain, nerve growth factor acts to enhance their survival, differentiation, and maintenance. In recent years, many additional neurotrophic factors have been discovered; some are structurally related to nerve growth factor while others are distinct from it. The robust actions of neurotrophic factors have suggested their use in preventing or lessening the dysfunction and death of neurons in neurologic disorders. We review the progress in defining neurotrophic factors and their receptors and in characterizing their actions. We also discuss some of the uses of neurotrophic factors in animal models of disease. Finally, we discuss how neurotrophic factors could be implicated in the pathogenesis of neurologic disorders.

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Neurotrophic factors are polypeptides that exert their actions through binding and activating specific cell surface receptors. It is increasingly apparent that neurotrophic factors have an important role in the growth, development, and maintenance of neurons in both the central and peripheral nervous systems. Evidence accumulated over the past few years points to the existence of a number of classes of neurotrophic factors and documents their remarkable potency on responsive neurons (Table 1). These data are enhancing and accelerating efforts to apply neurotrophic factors to the treatment of human neurologic disease. In this article we briefly discuss the neurotrophic factors most likely to be used in clinical studies in the near future. The structure and functions of these molecules and their receptors are reviewed. Thereafter we consider data from three experiments in which neurotrophic factors have been used in animal models of neurologic disease and the mechanisms by which neurotrophic factors may be implicated in neurodegeneration and its treatment.

TABLE 1.—*Neurotrophic Factors—A Partial Listing*

Factor	*Abbreviation*
Neurotrophins	
Nerve growth factor	NGF
Brain-derived neurotrophic factor	BDNF
Neurotrophin 3	NT-3
Neurotrophin 4/5	NT-4/5
Ciliary neurotrophic factor	CNTF
Leukemia inhibitory factor	LIF
Insulin and the insulinlike growth factors	IGF-I IGF-II
Tumor growth factor-β family	TGFβ
Glial cell line-derived neurotrophic factor	GDNF
Fibroblast growth factors	FGF-1 FGF-2 FGF-5
Epidermal growth factor	EGF

Neurotrophic Factors

Nerve Growth Factor and the Neurotrophins

The neurotrophic factors in the neurotrophin gene family consist of nerve growth factor (NGF), the best characterized member, brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5. Nerve growth factor was discovered by Levi-Montalcini and Hamburger in the late 1940s and early 1950s. They were examining the effect a target has on its innervating neural center and discovered that a particular mouse sarcoma evoked dramatic hypertrophy of innervating dorsal root ganglia and sympathetic ganglia. The authors noted substantial deviations from the normal pattern of embryonic development in the extent of ganglion hyperplasia, the involvement of ganglia distant from the site of the sarcoma, and the bizarre, excessive innervation of various viscera.[1] These findings led to the hypothesis that the neoplastic cells released a soluble, diffusible agent that promoted the differentiation and growth of sympathetic and sensory neurons. This idea was supported in experiments in which the mouse sarcoma elicited the same effects when transplanted onto the chorioallantoic membrane of chick em-

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ABBREVIATIONS USED IN TEXT

BDNF = brain-derived neurotrophic factor
cDNA = complementary DNA
CNTF = ciliary neurotrophic factor
GABA = γ-butyric acid
GDNF = glial cell line-derived neurotrophic factor
IGF-I, -II = insulinlike growth factors I, II
IL-6 = interleukin-6
LIF = leukemia inhibitory factor
mRNA = messenger RNA
NGF = nerve growth factor
NT-3, -4/5 = neurotrophins 3, 4/5

bryos, a location that allowed diffusion through the circulation but prevented direct contact between the sarcoma and developing tissue.[2] Levi-Montalcini and Hamburger called the diffusible agent nerve growth factor. They were later able to purify the material to homogeneity and discovered a rich source that permitted many subsequent investigations on its biology and chemistry.[3]

Nerve growth factor is a 118-amino acid polypeptide that acts on neurons in both the peripheral and central nervous systems. Responsive peripheral neurons include cells in the trigeminal sensory ganglion, dorsal root ganglia, and paravertebral sympathetic ganglia. Nerve growth factor also acts on adrenal chromaffin cells. In the central nervous system, two populations respond robustly to NGF. These are the cholinergic neurons of the basal forebrain and those of the caudate putamen.[3] Nerve growth factor activates responsive cells through binding to specific receptors on their surface.[4] One of these receptors is $p75^{NGFR}$. This glycosylated transmembrane protein binds NGF with low affinity. It appears to modulate NGF binding, but it is not yet clear how and to what extent it influences NGF signaling.[3] Another transmembrane glycoprotein, trkA, serves as a receptor for NGF. The intracellular domain of trkA encodes a tyrosine kinase (Figure 1). The binding of NGF to trkA causes it to dimerize; this activates its kinase and leads to autophosphorylation. Activating trkA kinase is necessary for transmitting the NGF signal.[4,5] Signal transduction is mediated through the phosphorylation of specific residues on trkA. These serve to bind and to activate other proteins, including phospholipase C-γ and phosphatidylinositol 3-kinase.[6,7] The signaling cascade produced by NGF is continued by a number of events, including the activation of other kinases and the generation of second messengers. The details of this cascade are only beginning to be discovered.

In both the peripheral and central nervous systems, NGF is produced in the target of innervating neurons.[3] There are clear examples where neurons have access to NGF mainly through contact with their targets. An interesting requirement imposed by this arrangement is the need to convey the NGF signal down the axon to the cell body. How this is achieved is not yet clear.[6] What is clear is that NGF signaling requires binding to trkA. In every case, NGF-responsive cells have been shown to bear trkA receptors. Thus, it appears that NGF produced in the target of developing and mature neurons binds to trkA on the neurites of these cells to initiate or maintain trophic relationships.

Brain-derived neurotrophic factor (BDNF) was discovered in 1982 as the culmination of a painstaking series of studies by Barde and colleagues.[8] It is a neurotrophic factor similar in structure to NGF. Indeed, the discovery of BDNF suggested the existence of a neurotrophin gene family. This neurotrophic factor is a 120-amino acid polypeptide that is 50% identical to NGF at the amino acid level. The sequence homology was used by several groups to isolate a third member of this family, neurotrophin 3 (NT-3),[9-13] and a fourth member, neurotrophin 4/5 (NT-4/5).[14-16] Cells in the peripheral nervous system that are responsive to BDNF include sensory neurons in the nodose ganglion and a subpopulation of dorsal root ganglia neurons. Brain-derived neurotrophic factor also acts on trigeminal mesencephalic neurons. Its targets in the central nervous system include retinal ganglion neurons, hippocampal neurons, basal forebrain cholinergic neurons, basal forebrain γ-aminobutyric acid (GABA)-ergic neurons, and GABAergic neurons of the ventral mesencephalon.[17,18] Recent studies have demonstrated that BDNF also acts on motoneurons.[19-22] This neurotrophic factor is found predominantly in the central nervous system, where it is most abundant in the adult. The highest levels of BDNF messenger RNA (mRNA) were found in the hippocampus, cortex, and cerebellum.[23] In situ hybridization histochemistry has localized BDNF mRNA to neurons in several brain regions. These include pyramidal, hilar, and dentate granule neurons of the hippocampus and neurons in the neocortex.[24] As with NGF, $p75^{NGFR}$

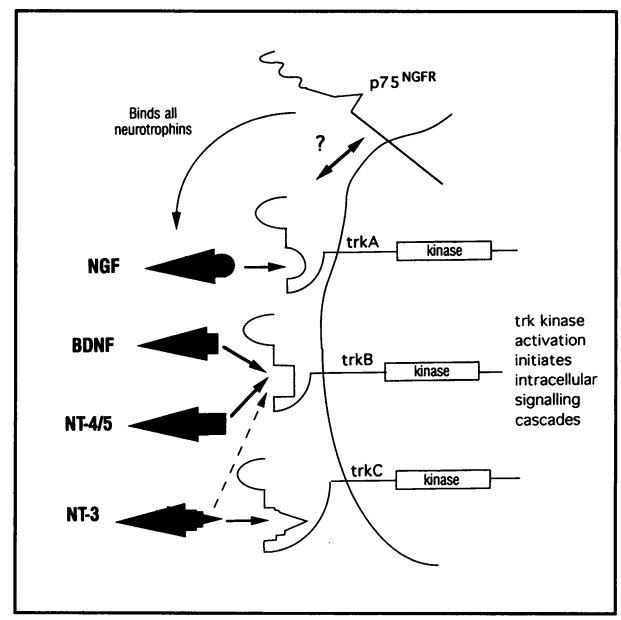

Figure 1.—The diagram illustrates the trk receptors and their specificities for the neurotrophins—nerve growth factor (**NGF**), brain-derived growth factor (**BDNF**), and neurotrophin (**NT**)-3 and NT-4/5. Ligand binding induces dimerization and autophosphorylation that activates signaling. The role of the low-affinity NGF receptor ($p75^{NGFR}$), which binds to all of the neurotrophins, is uncertain.

serves as a receptor for BDNF and binds this neurotrophic factor with low affinity. Another member of the trk gene family, trkB, is the signaling receptor for BDNF (Figure 1). Transcripts of trkB are found in many central nervous system regions, including hippocampus, basal forebrain, neocortex, ventral mesencephalon, and in spinal cord motoneurons.[6,18,24,25] There is an important distinction with respect to trkA and trkB: there are trkB transcripts that encode truncated proteins in which the tyrosine kinase domain is missing.[6] This finding raises the possibility that not all BDNF binding would lead to signaling.

Neurotrophin 3 is the third member of the neurotrophin family. Neurons responsive to NT-3 include cells in both the peripheral and central nervous systems. Among responsive peripheral cells are neurons of the dorsal root ganglia and nodose ganglion and possibly the sympathetic ganglia. In the central nervous system, NT-3-responsive cells include neurons of the trigeminal mesencephalic nucleus, hippocampus, and dopaminergic and GABAergic cells in the ventral mesencephalon.[17,18,26] Motoneurons of the spinal cord also respond.[21] In the peripheral nervous system, NT-3 mRNA is found in muscle. In the central nervous system, high levels of NT-3 mRNA are found in the hippocampus and cerebellum. Neurotrophin 3 mRNA has also been detected in the olfactory bulb, neocortex, diencephalon, midbrain, and spinal cord.[21,24] By in situ hybridization, NT-3 mRNA has been localized to neurons,[24] including embryonic motoneurons.[21,27] Like other neurotrophins, NT-3 binds to $p75^{NGFR}$. Interestingly, it acts by binding to yet another member of the trk family, trkC (Figure 1). Gene expression for trkC is widespread in the central nervous system, with high levels of trkC mRNA in the hippocampus, neocortex, and cerebellum.[28] Messenger RNA of trkC is also found in the ventral mesencephalon[18] and in motoneurons.[21] Like trkB, there are truncated and therefore presumably nonsignaling forms of trkC.[6]

Neurotrophin 4/5 is the most recently discovered member of the neurotrophin gene family. Cells in the peripheral nervous system that are responsive to NT-4/5 include trigeminal, dorsal root ganglion, jugular, sympathetic, and nodose ganglion neurons.[17,29] In the central nervous system, hippocampal neurons and dopaminergic and GABAergic neurons of the ventral mesencephalon respond,[18,26] as do motoneurons.[21] Messenger RNA of NT-4/5 is found in muscle and other peripheral tissues[16] and in the central nervous system. The highest amounts in the central nervous system were found in pons or medulla, hypothalamus, thalamus, and cerebellum.[24] Neurotrophin 4/5 is known to activate the trkB receptor (Figure 1).

The discovery of NGF as a target-derived trophic factor for peripheral neurons has had a major influence on studies directed at understanding its expression and actions in both the peripheral and central nervous systems. In general, data for the expression of NGF and trkA indicate that NGF produced in the target of developing and mature neurons acts through trkA to maintain the survival and enhance the differentiation of responsive neurons. The situation may be more complex for the other neurotrophins. In some instances, a target-derived trophic relationship can be inferred. In others, it appears that autocrine or paracrine relationships may hold. An example of the latter is found in the hippocampus, where it has been shown that certain hippocampal neurons contain mRNA of both BDNF and trkB.[24] A further complication is the production of truncated versions of both trkB and trkC. It will be interesting to further define and characterize the trophic relationships that neurotrophins use to influence the survival and function of their responsive cells.

Ciliary Neurotrophic Factor

During the past few years exciting developments have occurred with respect to another neurotrophic factor called ciliary neurotrophic factor (CNTF). This factor was first purified as a substance extracted from intraocular tissues that supported the survival of ciliary ganglion neurons.[30] In its activity on ciliary neurons and by physicochemical characteristics, the partially purified factor was found to be distinct from NGF. An enriched source of CNTF was found in the adult rat sciatic nerve, and a modified purification protocol was used to purify CNTF activity from this source.[30] This allowed sequencing studies to be done, and eventually CNTF was cloned in several laboratories.[31-33] As predicted from the complementary DNA (cDNA) sequences in rats, rabbits, and humans, CNTF is a protein of 200 amino acids with a molecular weight of 22,700. The availability of purified recombinant protein considerably enhanced studies of the biologic activity of CNTF. Responsive cells of the peripheral nervous system include ciliary ganglion neurons, dorsal root ganglia neurons, sympathetic neurons, and adrenal chromaffin cells. In the central nervous system, hippocampal neurons, forebrain cholinergic neurons, and other populations, including spinal cord motoneurons, are responsive.[17,30] The survival of motoneurons is enhanced by CNTF both in vitro and in vivo.[34-36] In recent studies, disruption of the CNTF gene was shown to produce progressive atrophy and loss of motoneurons.[37] Interestingly, oligodendroglial progenitor cells, referred to as 02A cells, respond to CNTF, as do mature oligodendrocytes.[38]

As indicated, peripheral nerves serve as a rich source for CNTF. The production of CNTF in Schwann cells explains the levels present.[30] An interesting feature of CNTF is the absence of a signal sequence. The meaning of this finding is uncertain, but the suggestion has been made that CNTF release may normally require cell injury with membrane disruption. Ciliary neurotrophic factor-like activity can be shown in stumps affixed to a lesioned nerve in the first few days after a nerve lesion.[30] In the central nervous system astrocytes may produce CNTF,[39] and the levels of production may increase after injury. Thus, it has been suggested that CNTF may play the role of a lesion factor.[30]

The actions of this neurotrophic factor include enhanced neuronal survival. This has been most dramatically demonstrated in studies in vivo. Axotomy of the facial nerve of newborn rats normally results in a substan-

tial loss of motoneurons in the facial nucleus. The application of CNTF to the proximal stump prevented the loss of most neurons.[35] Ciliary neurotrophic factor enhances the cholinergic differentiation of neonatal sympathetic neurons, an effect that parallels the actions of another neurotrophic factor called leukemia inhibitory factor (LIF).[30] These two factors are biochemically distinct, but activate similar receptors (discussed later). An interesting feature of CNTF action in the central nervous system is its ability to prevent the death of axotomized medial septal neurons, both cholinergic and noncholinergic in type. Interestingly, although CNTF promoted the survival of cholinergic neurons that are marked by $p75^{NGFR}$, it did not maintain the activity of choline acetyltransferase, its neurotransmitter synthetic enzyme.[30]

Experiments to identify the CNTF receptor led to the discovery that a specific CNTF binding protein, designated CNTF receptor α (CNTFRα), was homologous to a receptor for the hematopoietic cytokine, interleukin-6 (IL-6).[40] This finding led to several developments. The first was a structural analysis that suggested that IL-6 and CNTF were themselves distantly related and members of a larger family of cytokines that includes LIF, granulocyte colony-stimulating factor, and oncostatin M. All of these factors were known to activate similar intracellular signaling events, and there was the possibility that they used similar receptor systems. Subsequently it was found that CNTF, IL-6, LIF, and oncostatin M do share signal transducing receptor components (Figure 2).[40] All of them use gp130, a transmembrane protein initially identified as the IL-6 signal transducer. In addition, CNTF, LIF, and oncostatin M require a second signal transducer known as LIFRβ. Specificity of CNTF actions arises from its binding to the α-receptor component, together with LIFRβ and gp130. Receptor activation arises from the formation of a heterodimer between LIFRβ and gp130 (Figure 2). The signal is transduced through the activation of a family of cytoplasmic tyrosine kinases known as the Jak-Tyk family. Activation of these kinases leads to the initiation of the signaling cascade in the responsive cell, which includes the phosphorylation of other cellular proteins.[41] One of these is called acute-phase response factor. Acute-phase response factor is a latent cytoplasmic transcription factor that is rapidly activated in response to CNTF.[42]

Distribution of the CNTFRα receptor should define possible sites of CNTF action. In fact, through in situ hybridization histochemistry, CNTFRα has been localized to all the known neuronal targets of CNTF, including neurons in the peripheral ganglia and spinal cord motoneurons.[40] An interesting possibility arises from the structure of CNTFRα. This is a glycosylphosphatidylinositol-linked protein. This linkage is susceptible to enzymatic cleavage, and soluble CNTFRα can be found in cerebrospinal fluid.[40] It is known that cells bearing gp130 and LIFRβ that are normally not responsive to CNTF can be induced to respond by adding CNTF with soluble

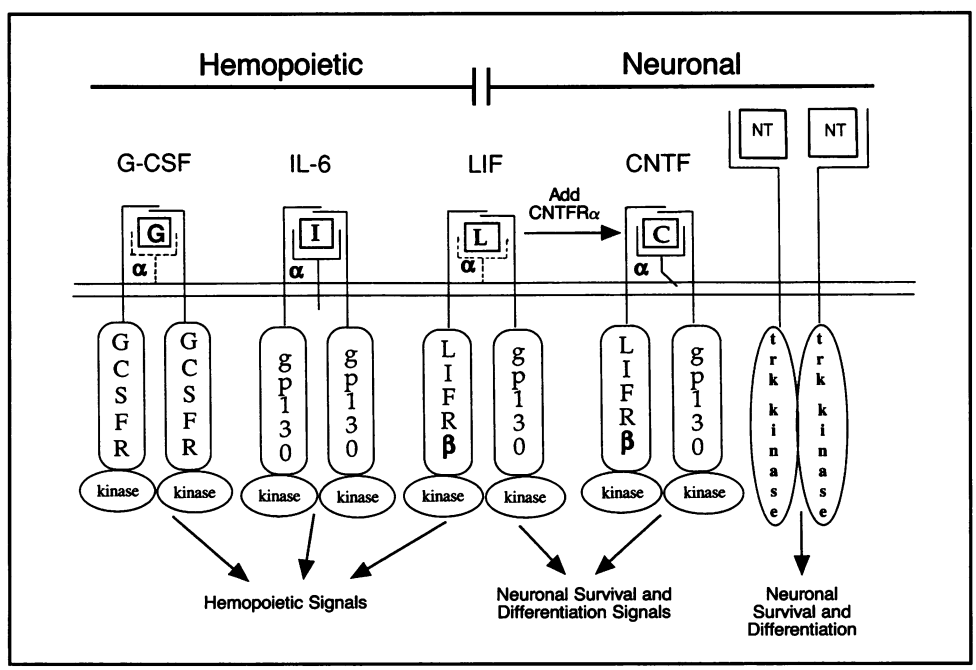

Figure 2.—Models are shown of granulocyte colony-stimulating factor (**G-CSF [G]**), interleukin-6 (**IL-6 [I]**), leukemia inhibitory factor (**LIF [L]**), and ciliary neurotrophic factor (**CNTF [C]**) receptor complexes (GCSFR, gp130, LIFRβ, and CNTFRα) compared with the trk receptor tyrosine kinases used by neurotrophins (**NT**) (adapted from Ip and Yancopoulos[40]). The cytokine receptors are depicted as either homodimers or heterodimers of β (gp130 and LIFRβ) components. Complex formation, including the dimerization of β-subunits, only occurs in response to ligand, and results in activation of associated Jak-Tyk tyrosine kinases. In the model, CNTFRα converts a functional LIF receptor complex into a functional CNTF receptor complex. Possible α components for G-CSF and LIF receptor complexes are indicated by dashed lines.

CNTFRα.[43] This suggests two possibilities. First, following the release of CNTFRα from the neuronal membrane, it could act to bind CNTF and prevent it from binding to its receptor on neurons. Alternatively, after binding to CNTF, CNTFRα could carry it to cells expressing gp130 and LIFRβ and induce a response. This analysis suggests a complexity and spectrum of CNTF actions that may be different from those of the neurotrophins.

Other Growth Factors of Interest

The insulin gene family comprises the genes for insulin and the insulinlike growth factors I and II (IGF-I and IGF-II). There is increasing interest in the actions of these molecules in the nervous system. Insulinlike growth factor I is of considerable interest. The mRNA for IGF-I is present in the target zones of trigeminal and sympathetic neurons during the innervation period. It is also found in projection neurons in the maturing sensory and cerebellar relay systems and in nonpyramidal cells of the hippocampus and cerebral cortex. It may also be synthesized by Purkinje cells in the developing cerebellum.[44] There is regulation of IGF-I mRNA levels during development with differences from region to region in the brain. Support for an IGF-I role in developmental regulation of neuronal function and survival comes from in vitro studies showing that IGF-I can induce neurite outgrowth in cultured motor, sensory, and sympathetic neurons. In addition, cortical neurons have been shown to respond, and IGF-I induces oligodendrocyte differentiation and myelin synthesis.[44] Suggesting a role for IGF-I in the development of the neuromuscular junction, IGF-I mRNA levels increase in muscle between embryonic day 14 and birth. Postnatal inhibition of IGF-I mRNA may contribute to the elimination of polyneuronal innervation.[45]

Insulin and the IGFs elicit their actions by binding to specific receptors on the surface of responsive cells. The receptors for insulin and IGF-I are similar.[44] Each is a disulfide-linked heterotetramer consisting of two α- and two β-subunits. The two α-subunits bind the ligand through a cystein-rich domain. These subunits lie entirely outside the cell. The β-subunits, which are linked by disulfide bridges to the α-subunits, contain a short extracellular domain and a transmembrane segment, followed by a cytoplasmic domain. The cytoplasmic domain contains an adenosine triphosphate-binding site and a tyrosine kinase domain. These receptors are formally similar to those defined earlier for the neurotrophins. Activation of the receptor occurs through ligand binding to the α-subunit with a subsequent conformational change in the β-subunits leading to receptor tyrosine kinase activation and autophosphorylation. This is followed by the activation of various intracellular substrates through phosphorylation and the initiation of a cascade of events leading to the biologic response. The IGF-II receptor is distinct from that for insulin and IGF-I. The IGF-II receptor is a single polypeptide chain with a large extracellular domain and a short cytoplasmic domain. Signaling through this receptor may involve glycine protein activation. The concentration of IGF-I receptors peaks in the brain during fetal development. Examining the postnatal and mature brain, IGF-I receptor mRNA is found in sensory and cerebellar relay systems, in the frontal cortex, Ammon's horn, the amygdaloid nuclei, and the suprachiasmatic nucleus. Autoradiography for IGF-I receptors shows rather dense labeling in the olfactory bulb, cerebellum, choroid plexus, and other sites including neocortex.[44] Thus, both IGF-I and its receptor are widely distributed.

Recently a glial cell line-derived neurotrophic factor (GDNF) for midbrain dopaminergic neurons has been described.[46] This potent factor was secreted by a rat glial cell line. It was purified to homogeneity and shown to promote dopamine uptake in cultures of neurons from the midbrain. Both rat and human cDNA for GDNF were cloned using probes based on the amino terminal sequence of purified GDNF. The protein predicted from the sequence data is a secreted molecule of 134 amino acids. It apparently exists as a disulfide-bonded homodimer and is distantly related to members of the transforming growth factor-β superfamily. In midbrain cultures, GDNF promoted the survival and differentiation of dopaminergic neurons. These effects appeared to be relatively specific in that GDNF did not act on GABAergic or on serotonergic neurons.[46]

Animal Models of Disease Treated by Neurotrophic Factors

The remarkable ability of neurotrophic factors to stimulate the survival and differentiation of neurons has suggested their use as trophic agents in diseases. Given advances in understanding the actions of these factors and the distribution of their receptors, we can now begin to develop a list of neurologic disorders and a corresponding list of factors that may prove useful (Table 2). Important diseases that might respond to neurotrophic factors are Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Peripheral neuropathy also suggests itself. Clinical trials are currently underway for amyotrophic lateral sclerosis and vincristine-induced peripheral neuropathy. At the same time, ongoing experiments in animal models of disease encourage the view that neurotrophic factors can be used to treat neurologic disorders.

Nerve Growth Factor Treatment of Taxol Neuropathy

Interesting studies have been done on NGF effects on the toxic neuropathy produced by the chemotherapeutic agent taxol.[47] Taxol has been used against solid tumors such as malignant melanoma and ovarian carcinoma. Unfortunately, its use induces a toxic sensory neuropathy, and this phenomenon has limited its clinical usefulness. The effects of NGF on sensory neurons both in vivo and in vitro encouraged a study in which this trophic factor was used to attempt to prevent taxol neuropathy in vivo.

For these studies an animal model for taxol neuropathy in mice was established. Taxol was administered to the mice intraperitoneally for six days, and they were tested three days thereafter for changes in sensory function. To assess thermal pain threshold, a modification of a

TABLE 2.—*Neurologic Disorders and Candidate Neurotrophic Factors for Therapy*

Disorder	*Neurotrophic Factor**
Peripheral neuropathy	
Sensory neurons	NGF, BDNF, NT-3, NT-4/5
Sympathetic neurons	NGF, FGF-2
Parasympathetic neurons	CNTF
Amyotrophic lateral sclerosis	
Motoneurons	CNTF, BDNF, NT-4/5, IGF-1
Alzheimer's disease	
Basal forebrain cholinergic neurons	NGF
Neocortical neurons	BDNF, NT-3, NT-4/5
Hippocampal neurons	BDNF, NT-3, NT-4/5
Parkinson's disease	
Dopaminergic neurons	GDNF, BDNF, NT-4/5, FGF-1, FGF-2, IGF-I
Huntington's disease	
Striatal interneurons	BDNF, NT-4/5
Striatal cholinergic neurons	NGF

*See Table 1 for abbreviations of neurotrophic factors.

standard tail-flick test was used. Mice were partially restrained, and their tails were placed in a beaker of water. The temperature of the water was increased in increments, and the experimenters observed the temperature at which the mouse flicked its tail out of the water. They were able to show that taxol significantly increased the temperature at which tail-flick occurred. When NGF was administered with taxol, there was no significant difference from control animals in tail-flick temperature. Administering taxol was also found to reduce the mean level of substance P in the dorsal root ganglia. Substance P marks a portion of the NGF responsive neurons in the ganglia. Remarkably, NGF prevented the taxol-mediated decrease in substance P levels. Electrophysiologic studies were also carried out, and compound sensory amplitudes were depressed in the taxol-treated subjects. The coadministration of NGF prevented the decrease. There was little if any effect of taxol on the distal latency recorded in the caudal nerve.[47]

These studies demonstrate clearly that NGF can prevent the effects of taxol on measures of sensory nociceptive function. How NGF exerts its effects is uncertain. One possibility is that it in some way directly counteracts taxol's effects. Another possibility is that NGF protects neurons through some indirect means. An interesting possibility raised by investigators is that taxol's effects on the cytoskeleton may in some way inhibit retrograde transport of the signal normally arising from NGF binding to its receptors in the periphery of sensory neurons.[47] If this were the case, then the administration of exogenous nerve growth factor could correct this deficiency by binding to receptors on or near the cell body. These studies suggest that NGF may be used to limit the dose-related toxicity of taxol and thus to facilitate the use of this drug in cancer patients. Conceivably, NGF or other neurotrophic factors acting on peripheral neurons could be used to augment the clinical usefulness of other cancer chemotherapeutic agents that produce peripheral nerve injury.

Ciliary Neurotrophic Factor and Motoneuron Disease

Several mouse mutants have been used to model motoneuron disease. The pmn/pmn mouse is an autosomal recessive mutant with progressive motor neuronopathy.[48] In homozygotes, paralysis of the hind limbs begins during the third week of life. The forelimbs become weak thereafter, and all mice die between six and seven weeks following birth. Histologic study of muscle shows neurogenic atrophy. Axonal degeneration is seen, which appears to start at motor end plates; it is found predominantly in sciatic nerve and its branches and in the phrenic nerve. Unaffected nerve fibers are normally myelinated, and sensory axons are spared. In the ventral horns, motoneurons show a reduction in cell size, then chromatolysis, and finally cell death. The gene responsible for this disorder is unknown.

On the basis of CNTF effects on motoneurons in vivo and in vitro, including the ability of this factor to prevent lesion-mediated degeneration of rat motoneurons during early postnatal life,[35] investigators pursued the hypothesis that CNTF could be used to prevent motoneuron degeneration in pmn/pmn mice.[49] It was first demonstrated that abnormalities in CNTF production were unlikely to cause the disease. Messenger RNA levels of CNTF in the sciatic nerve of these subjects were similar to those in normal mice. Also, extracts of nerves showed an equivalent amount of CNTF biologic activity. To administer CNTF to these subjects, a stable cell line was created by a transfection of mouse D3 cells with a construct in which CNTF genomic DNA was cloned 3′ to a cDNA fragment coding for the first 20 amino acids of the mouse prepro/NGF sequence, which includes the entire signal peptide for this molecule. The thought was that this construct would allow both synthesis and secretion of CNTF in these animals. Experiments in which CNTF expression was tested in vitro showed that CNTF activity was readily released into the culture medium. After intraperitoneal

administration of these cells on postnatal day 21, intraabdominal tumors were produced, and it was shown that CNTF was released into the serum of these animals. The production of CNTF in vivo was associated with enhanced survival and with enhanced motor performance compared with untreated or mock-treated pmn/pmn mice. Both treated and untreated mice were killed between postnatal days 40 and 48. Examination of the facial nucleus showed that the number of motoneurons was greatly decreased in pmn/pmn mice and that treatment with CNTF had a large protective effect. Thus, whereas in untreated mice only 60% of neurons remained, in treated mice, 86% of cells were present. Also, the number of axons in the phrenic nerve was substantially increased by CNTF treatment.[49]

The data show that CNTF rescues motoneurons from degeneration in pmn/pmn mice. They suggest that CNTF might be used to prevent motoneuron death and dysfunction in motoneuron disease in humans. The possibility is raised that other factors active on motoneurons might also be useful, either given separately or in conjunction with CNTF.

Nerve Growth Factor and the Trisomy 16 Mouse

The focus of work in our laboratory has been on understanding the natural processes of normal and diseased basal forebrain cholinergic neurons. Special emphasis has been placed on the response of these neurons to neurotrophic factors, especially NGF. Basal forebrain cholinergic neurons atrophy and die in patients with Alzheimer's disease, and it is known that these neurons are likely to contribute substantially to learning and memory deficits in these patients.[50] In developing an animal model for Alzheimer's disease, we made note of the interesting observation that the neurologic abnormalities of Alzheimer's disease universally develop at an early age in patients with the Down syndrome (trisomy 21). We reasoned that an animal model for Down syndrome might recapitulate important features of the neuropathogenesis of Alzheimer's disease and allow us to pursue the mechanism for the degeneration of basal forebrain cholinergic neurons. The trisomy 16 mouse is a genetic model for human Down syndrome. Mouse chromosome 16 contains a cluster of genes and loci that are also located on the proximal arm of human chromosome 21. These include the amyloid precursor protein, one of the glutamate receptor genes, and superoxide dismutase 1. Fetal trisomy 16 mice demonstrate phenotypic features seen in Down syndrome, including endocardial cushion defects and hematologic and immunologic abnormalities.

We hypothesized that cholinergic neurons of trisomy 16 murine basal forebrain would show degenerative changes over time in vivo. To compare cholinergic neurons from trisomy 16 and from normal mice, we transplanted fetal basal forebrain from trisomy 16 mice and from control fetuses into the hippocampus of normal mice. The hippocampus is the normal target of these neurons. Transplantation experiments showed that both trisomy 16 and control grafts survived for long periods in vivo. Interestingly, while cholinergic neurons appeared normal in both trisomy 16 and control transplants after one month, after six months there was clear-cut atrophy of trisomy 16 neurons. Atrophy was specific for cholinergic neurons.[51] To determine whether NGF could reverse this atrophy, NGF was administered by intraventricular cannulae between 5½ and 6 months of age. Vehicle injection served as the control. Animals were killed at 6 months of age, and grafts were assessed by immunostaining for choline acetyltransferase, the neurotransmitter synthetic enzyme for cholinergic neurons. As in earlier studies, there was atrophy of trisomy 16 cholinergic neurons relative to control neurons. The mean cross-sectional area was reduced by about 20% in vehicle-treated subjects. Following NGF infusion, trisomy 16 cholinergic neurons were significantly enlarged. Remarkably, both trisomy 16 and control neurons treated with NGF were substantially larger than control cholinergic neurons in vehicle-treated animals. The increase for trisomy 16 cells was to a size 29% greater than vehicle-treated control cholinergic neurons. There was no significant difference between the size of NGF-treated trisomy 16 and control neurons.[52] In follow-up studies we asked whether NGF levels were different in the hippocampus of animals that received trisomy 16 cells versus those that received control cells. No difference in NGF levels was apparent. Indeed, the size of the host basal forebrain cholinergic neurons, which have their axons in the same hippocampal territories as transplanted cells, was normal on both the side of the trisomy 16 transplant and the side of the control transplant. This suggests that the level of NGF available to trisomy 16 and control neurons was equivalent. We next asked whether NGF receptors continued to be expressed in neurons of trisomy 16 mice. We found evidence of both trkA and $p75^{NGFR}$ expression in both control and trisomy 16 neurons.[52] The levels of receptor expression and binding are now being addressed.

Using the trisomy 16 mouse model of the Down syndrome, we have shown that NGF can reverse genetically determined neuronal atrophy. Further analysis of this model may give insight into pathways that lead to neuronal degeneration in vivo and establish how NGF and other neurotrophic factors could be used to prevent dysfunction and death of these neurons in Alzheimer's disease.

Pursuing the Mechanisms of Neurodegeneration

The studies cited in this article give evidence that neurotrophic factors can prevent or reverse the degeneration and dysfunction of neurons caused by environmental and genetic insults. In addition to suggesting novel forms of therapy for these disorders, they raise the question of whether neurotrophic factors may be implicated in the pathogenesis of nervous system disorders. One possibility is that a primary deficiency of the neurotrophic factor is at the root of the disease. The examples cited earlier provide evidence that this is not required to produce such disorders. Indeed, in the case of the trisomy 16 mouse

transplants, atrophy occurred over a several-month period in vivo in the presence of what appear to have been normal levels of NGF. Thus, it would appear that an intrinsic abnormality in trisomy 16 cells was responsible for the changes that were demonstrated. No abnormality of a trophic factor has yet been clearly implicated in a disease state. Nevertheless, recent exciting work on mice in which the genes for either NGF or BDNF have been knocked out show unequivocally that eliminating the production of individual neurotrophic factors can produce considerable neuronal loss.[53,54]

A second possible explanation for neurodegeneration is that there is some fundamental abnormality in the receptor for a specific neurotrophic factor. Again, there are no disease examples to prove the case, but the possibility can be easily envisioned. A third possibility is that events downstream from the receptor that are important for signaling that leads to a maintenance of neuronal survival and function are altered in diseased neurons. In fact, these two possibilities can be combined in the case of the trisomy 16 mouse to suggest a means by which these neurons degenerate. It is known that NGF must bind to these receptors to activate its responsive cells. It is known also that one response to NGF is for gene expression for its receptors to be induced in these neurons.[55] Thus, even a subtle decrease in the ability of NGF receptors to be bound or activated by NGF, or in any of the many downstream events leading to the regulation of receptor gene expression, could in time result in fewer receptors at the cell surface. This situation could create progressive neuronal atrophy and conceivably even death of these cells in the presence of normal levels of NGF.

Finally, it is worth mentioning that the response of neurons to neurotrophic factors could be substantially different in patients with disease. The possibility can be envisioned that both beneficial and deleterious effects could arise from neurotrophic factor treatment of diseased neurons. Although it will be difficult to predict in advance in which diseases this may occur, these observations argue strongly for carrying out studies in realistic animal models of disease before human trials are conducted. In the case of the trisomy 16 mouse, NGF treatment did not appear to injure the responding neurons and created no new disorder.[52] Thus, for NGF there is no evidence that a deleterious effect would accompany its actions on diseased basal forebrain cholinergic neurons.

The observations cited in this article make it clear that there is a great deal of work yet to do to understand and to exploit the therapeutic potential of neurotrophic factors. Studies on the biology of these factors must proceed concurrently with studies designed to understand the mechanism of their action in reversing neurodegeneration.

Clinical Trials Using Neurotrophic Factors

There are several ongoing trials in which neurotrophic factors are being used to attempt to inhibit the dysfunction and death of neurons in specific neurologic disorders. Genentech has sponsored studies examining NGF for the treatment of peripheral neuropathy. A Phase I trial is now complete, and there are plans for testing NGF effects in diabetic neuropathy later this year. Genentech has also announced an interest in sponsoring trials of NGF in Alzheimer's disease. The neurotrophic factor would be delivered by intraventricular cannula. This ambitious trial is scheduled to begin within the next year. Amgen-Regeneron Partners are sponsoring a project to examine the effects of BDNF in amyotrophic lateral sclerosis. Other trials of neurotrophic factors have also been directed at this neurologic disorder. A Regeneron-sponsored Phase III trial using CNTF at either a high or a low dose was recently completed. The final analysis of the data is pending, but based on an interim analysis, it is not expected that CNTF-treated patients will be improved relative to placebo-treated controls. This is thought to be due, at least in part, to impaired neuromuscular function resulting from untoward side effects, which included anorexia, weight loss, nausea, and cough. A second trial for CNTF in amyotrophic lateral sclerosis is being sponsored by Synergen. In this trial, CNTF is being used at a lower dose. There are no results as yet available. Cephalon is also sponsoring trials in this disorder, using IGF-I in a Phase II/III North American trial that should be completed by the end of 1994. Preliminary data analysis is expected to be available in early 1995. Cephalon is sponsoring a similar trial in Europe; preliminary data should be available by mid- to late-1995. Finally, Cephalon is planning a Phase II trial using IGF-I in vincristine neuropathy.

REFERENCES

1. Levi-Montalcini R, Hamburger V: Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool 1951; 116:321-362

2. Levi-Montalcini R, Hamburger V: A diffusible agent of mouse sarcoma, producing hyperplasia of sympathetic ganglia and hyperneurotization of viscera in the chick embryo. J Exp Zool 1953; 123:233-288

3. Longo FM, Holtzman DM, Grimes ML, Mobley WC: Nerve growth factor: Actions in the peripheral and central nervous systems, *In* Loughlin S, Fallon J (Eds): Neurotrophic Factors. San Diego, Calif, Academic Press, 1993, pp 209-256

4. Chao MV: Neurotrophin receptors: A window into neuronal differentiation. Neuron 1992; 9:583-593

5. Kaplan DR, Hempstead BL, Martin-Zanca D, Chao MV, Parada LF: The trk proto-oncogene product: A signal transducing receptor for nerve growth factor. Science 1991; 252:554-558

6. Grimes M, Zhou J, Li Y, Holtzman D, Mobley WC: Neurotrophin signalling in the nervous system. Semin Neurosci 1993; 5:239-247

7. Saltiel AR, Ohmichi M: Pleiotropic signaling from receptor tyrosine kinases. Curr Opin Neurobiol 1993; 3:352-359

8. Barde YA, Edgar D, Thoenen H: Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982; 1:549-553

9. Ernfors P, Ibanez CF, Ebendal T, Olson L, Persson H: Molecular cloning and neurotrophic activities of a protein with structural similarities to beta-NGF: Developmental and topographical expression in the brain. Proc Natl Acad Sci USA 1990: 87:5454-5458

10. Hohn A, Leibrock J, Bailey K, Barde YA: Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 1990; 344:339-341

11. Maisonpierre PC, Belluscio L, Squinto S, et al: Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. Science 1990; 247:1446-1451

12. Rosenthal A, Goeddel DV, Nguyen T, et al: Primary structure and biological activity of a novel human neurotrophic factor. Neuron 1990; 4:767-773

13. Jones KR, Reichardt LF: Molecular cloning of a human gene that is a member of the nerve growth factor family. Proc Natl Acad Sci USA 1990; 87:8060-8064

14. Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A: Neurotrophin-5: A novel neurotrophic factor that activates trk and trk B. Neuron 1991; 7:857-866

15. Hallböök F, Ibanez C, Persson H: Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron 1991; 6:845-858

16. Ip NY, Ibanez CF, Nye SH, et al: Mammalian neurotrophin 4: Structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci USA 1992; 89:3060-3064

17. Korsching S: The neurotrophic factor concept: A reexamination. J Neurosci 1993; 13:2739-2748

18. Hyman C, Juhasz M, Jackson C, et al: Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. J Neurosci 1994; 14:335-347

19. Yan Q, Elliott J, Snider WD: Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. Nature 1992; 360:753-755

20. Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA: Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature 1992; 360:757-759

21. Henderson CE, Camu W, Mettling C, et al: Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature 1993; 363:266-270

22. Koliatsos VE, Clatterbuck RE, Winslow JW, Cayouette MH, Price DL: Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. Neuron 1993; 10:359-367

23. Lindsay RM: Brain-derived neurotrophic factor: An NGF-related neurotrophin, *In* Loughlin S, Fallon J (Eds): Neurotrophic Factors. San Diego, Calif, Academic Press, 1993, pp 257-284

24. Persson H: Neurotrophin production in the brain. Semin Neurosci 1993; 5:227-237

25. Ringstedt T, Lagercrantz H, Persson H: Expression of members of the trk family in the developing postnatal rat brain. Dev Brain Res 1993; 72:119-131

26. Ip NY, Li Y, Yancopoulos GD, Lindsay RM: Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. J Neurosci 1993; 13:3394-3405

27. Schecterson LC, Bothwell M: Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. Neuron 1992; 9:449-463

28. Lamballe F, Klein R, Barbacid M: trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 1991; 66:967-979

29. Davies AM, Horton A, Burton LE, Schmelzer C, Vandlen R, Rosenthal A: Neurotrophin-4/5 is a mammalian-specific survival factor for distinct populations of sensory neurons. J Neurosci 1993; 13:4961-4967

30. Manthorpe M, Louis JC, Hagg T, Varon S: Ciliary neurotrophic factor, *In* Loughlin S, Fallon J (Eds): Neurotrophic Factors. San Diego, Calif, Academic Press, 1993, pp 443-473

31. Lin LF, Mismer D, Lile J, et al: Purification, cloning, and expression of ciliary neurotrophic factor (CNTF). Science 1989; 246:1023-1025

32. Stockli KA, Lottspeich F, Sendtner M, et al: Molecular cloning, expression and regional rat ciliary neurotrophic factor. Nature (London) 1989; 342:920-923

33. Lam A, Fuller F, Miller J, et al: Sequence and structural organization of the human gene encoding ciliary neurotrophic factor. Gene 1991; 102:271-276

34. Arakawa Y, Sendtner M, Thoenen H: Survival effect of ciliary neurotrophic factor (CTNF) on chick embryonic motoneurons in culture: Comparison with other neurotrophic factors and cytokines. J Neurosci 1990; 10:3507-3515

35. Sendtner M, Kreutzberg GW, Thoenen H: Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. Nature 1990; 345:440-441

36. Oppenheim RW, Prevette D, Qin-Wei Y, Collins F, MacDonald J: Control of embryonic motoneuron survival in vivo by ciliary neurotrophic factor. Science 1991; 251:1616-1618

37. Masu Y, Wolf E, Holtmann B, Sendtner M, Brem G, Thoenen H: Disruption of the CNTF gene results in motor neuron degeneration. Nature 1993; 365:27-32

38. Louis JC, Magal E, Takayama S, Varon S: CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. Science 1993; 259:689-692

39. Stockli KA, Lillien LE, Naher-Noe M, et al: Regional distribution, developmental changes, and cellular localization of CNTF-mRNA and protein in the rat brain. J Cell Biol 1991; 115:447-459

40. Ip NY, Yancopoulos GD: Receptors and signaling pathways of ciliary neurotrophic factor and the neurotrophins. Semin Neurosci 1993; 5:249-257

41. Stahl N, Boulton TG, Farruggella T, et al: Association and activation of Jak-Tyk kinases by CNTF-LIF-IL-6 β-receptor components. Science 1994; 263:92-95

42. Lutticken C, Wegenka UM, Yuan J, et al: Association of transcription factor APRF and protein kinase Jak 1 with the interleukin-6 signal transducer gp130. Science 1994; 263:89-92

43. Davis S, Aldrich TH, Ip NY, et al: Released form of CNTF receptor α component as a soluble mediator of CNTF responses. Science 1993; 259:1736-1739

44. LeRoith D, Roberts CTJ, Werner H, et al: Insulin-like growth factors in the brain, *In* Loughlin S, Fallon J (Eds): Neurotrophic Factors. San Diego, Calif, Academic Press, 1993, pp 391-414

45. Ishii DN: Neurobiology of insulin and insulin-like growth factors, *In* Loughlin S, Fallon J (Eds): Neurotrophic Factors. San Diego, Calif, Academic Press, 1993, pp 415-442

46. Lin LFH, Doherty DH, Lile JD, Bektesh S, Collins F: GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 1993; 260:1130-1132

47. Apfel SC, Lipton RB, Arezzo JC, Kessler JA: Nerve growth factor prevents toxic neuropathy in mice. Ann Neurol 1991; 29:87-90

48. Schmalbruch H, Jensen HJS, Bjaerg M, Kamieniecka Z, Kurland L: A new mouse mutant with progressive motor neuronopathy. J Neuropathol Exp Neurol 1991; 50:192-204

49. Sendtner M, Schmalbruch H, Stockli KA, et al: Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. Nature 1992; 358:502-504

50. Coyle JT, Price DL, DeLong MR: Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 1983; 219:1184-1190

51. Holtzman DM, Li Y, DeArmond SJ, et al: A mouse model of neurodegeneration: Atrophy of basal forebrain neurons in Ts 16 transplants. Proc Natl Acad Sci USA 1992; 89:1383-1387

52. Holtzman DM, Li Y, Chen K, et al: Nerve growth factor reverses neuronal atrophy in a Down syndrome model of age-related neurodegeneration. Neurology 1993; 43:2668-2673

53. Crowley C, Spencer SD, Nishimura MC, et al: Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 1994; 76:1001-1011

54. Jones KR, Fariñas I, Backus C, Reichardt LF: Targeted disruption of the BDNF gene perturbs brain and sensory neuron development. Cell 1994; 76:989-999

55. Holtzman DM, Li Y, Parada LF, et al: $p140^{trk}$ mRNA marks NGF-responsive forebrain cholinergic neurons: Evidence that trk gene expression is induced by NGF. Neuron 1992; 9:465-478